Breaking Barriers in the Genomics and Pharmacogenetics of Drug Addiction

MK Ho[1], D Goldman[2], A Heinz[3], J Kaprio[4], MJ Kreek[5], MD Li[6], MR Munafò[7] and RF Tyndale[1]

Drug addiction remains a substantial health issue with limited treatment options currently available. Despite considerable advances in the understanding of human genetic architecture, the genetic underpinning of complex disorders remains elusive. On the basis of our current understanding of neurobiology, numerous candidate genes have been implicated in the etiology and response to treatment for different addictions. Genome-wide association (GWA) studies have also identified novel targets. However, replication of these studies is often lacking, and this complicates interpretation. The situation is expected to improve as issues such as phenotypic characterization, the apparent "missing heritability," the identification of functional variants, and possible gene–environment (G × E) interactions are addressed. In addition, there is growing evidence that genetic information can be useful in refining the choice of addiction treatment. As genetic testing becomes more common in the practice of medicine, a variety of ethical and practical challenges, some of which are unique to drug addiction, will also need to be considered.

Drug addictions are a set of neurobiologically connected, chronic, and relapsing medical and psychiatric diseases characterized by persistent and compulsive use despite significant harmful consequences. Continued use of the addictive agent results in neuroadaptation, with associated changes persisting long after use is discontinued. The World Health Organization estimates that there are currently 185 million users of illicit drugs, 1.3 billion tobacco smokers, and 2 billion alcohol users worldwide.[1] In addition to the often immense damage to the individual, the economic costs of drug addiction are substantial. In the United States, annual costs have been estimated at $181 billion for illicit drugs, $168 billion for tobacco, and $185 billion for alcohol; these estimates include burdens on the health and criminal systems as well as loss of productivity in the workforce.[2]

The pathogenesis of addiction involves a series of complex interactions among biological factors (e.g., genetic vulnerability, gender, physiological and behavioral response to drug experimentation and use, and drug-induced alterations in gene expression and resultant proteins), environmental factors (e.g., legality, acceptability, and availability), psychological factors (e.g., novelty seeking, harm avoidance, and personality traits), and drug use factors (e.g., dose, pattern of use, and route of administration). The voluntary initiation and continuation of a behavior that is harmful to health is an important aspect of the etiology of many common diseases, including cardiovascular disease and metabolic syndrome. However, in addictions, the role of volition in initiation and subsequent drug-induced impairments in judgment are most salient. Moreover, licit and illicit drug use are typically initiated in childhood, when the ability to balance the apparent short-term benefits of experimentation and use with the addictive potential and long-term physical and mental consequences of dependence is generally lacking. Very few adults initiate drug use voluntarily if they have remained drug naive into adulthood, and this is why tobacco and alcohol industries specifically target youth to recruit new users. However, abuse and addiction to prescription opiates are also becoming a problem among older adults. A strong genetic component has been identified in the etiologies of addictions, and several addictions are among the most heritable psychiatric disorders.[3] Numerous family, twin, and adoption studies have provided consistent evidence for the role of genetic factors, by estimating heritability as the fraction of interindividual differences that can be attributed to genetic differences between individuals. Estimates

[1]Centre for Addiction & Mental Health, Department of Psychiatry, Pharmacology and Toxicology, University of Toronto, Toronto, Ontario, Canada; [2]National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, USA; [3]Department of Psychiatry and Psychotherapy, Charité-Universitätsmedizin, Berlin, Germany; [4]Institute for Molecular Medicine and Department of Public Health, University of Helsinki, Helsinki, Finland and National Institute for Health and Welfare, Helsinki, Finland; [5]Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, New York, USA; [6]Department of Psychiatry and Neurobehavioral Sciences, University of Virginia School of Medicine, Charlottesville, Virginia, USA; [7]Department of Experimental Psychology, University of Bristol, Bristol, UK. Correspondence: RF Tyndale (r.tyndale@utoronto.ca)

Received 12 March 2010; accepted 16 June 2010; advance online publication 27 October 2010. doi:10.1038/clpt.2010.175

Table 1 Heritability estimates for different drugs of abuse

Phenotype	Heritability estimates
Smoking	
Persistence	28–84%
Cigarette consumption	45–86%
Nicotine dependence	31–75%
Nicotine withdrawal symptoms	26–48%
Smoking cessation	50–58%
Alcoholism	
Alcohol abuse/dependence	50–70%
Consumption levels	45–58%
Problem drinking	8–50%
Opiates/heroin	
Abuse and/or dependence	43–60%
Sedatives	
Abuse and/or dependence	29–58%
Psychostimulants	
Abuse and/or dependence	42–74%

These studies have been reviewed in greater detail elsewhere.[3,4,6,7,50] A list of the primary references can be found in **Supplementary Table S2** online.

of the heritability of smoking persistence/dependence vary from 0.4 to 0.8 (reviewed in refs. 4,5; **Table 1**), and estimates for the heritability of alcoholism typically range from 0.5 to 0.7 (reviewed in refs. 3,6; **Table 1**). Similarly, heritability estimates for initiation and/or dependence of illicit drug use have been reported to be 0.3–0.6, although fewer studies have focused on this (reviewed in refs. 3,7; **Table 1**). It should be noted that heritability estimates are population- and time-specific, and other factors that can influence the risk of addiction indirectly (such as impulsivity) are also heritable. Investigation of why heritability varies between populations and over time can provide insights into the role of novel environmental influences.

Despite drug- and drug-class-specific mechanisms of action and psychoactive effects, there is a substantial overlap of genetic factors that underlie addiction to most classes of drugs. For example, ~60% of genetic influences are shared between nicotine and alcohol dependence.[7] The notion of a shared biological component underlying addiction to different drugs of abuse is reflected in the high rates of comorbid dependence to different substances, similar patterns in the initiation and continuation of drug use, evidence of cross-tolerance and cross-dependence related to different substances, and common mechanisms underlying drug reward in the brain. Although the heritability of drug addictions has been determined by many twin and family studies, our understanding of the specific genes involved remains limited.

With the completion of a canonical sequence for the human genome in 2003, and very rapid advances in DNA sequencing technologies, the possibility that the sequencing of all three billion DNA base pairs will become routine in medical practice is not farfetched. The canonical human genome sequence was a 13-year effort involving DNA fragment cloning, shotgun sequencing of random DNA fragments, and advances in bioinformatics capabilities for sequence assembly, and it required the efforts of hundreds of people and machines at a cost of $3 billion. Using sequencing technologies in which up to a billion DNA fragments are simultaneously sequenced in a single run on one machine, a human genome can now be sequenced in several weeks for less than $50,000. The "$1,000 genome" is thought to be a reasonable goal within the next few years, and large-scale sequencing of human genomes (such as the 1000 Genomes Project) is already under way. The International HapMap Project, which was also established in 2003, identifies and catalogs all common human genetic variants with the goal of using this information to find the genes that affect health, diseases, and individual responses to medications and environmental factors. To date, some 22 million polymorphisms are known, and the frequencies of alleles and linkage disequilibrium relationships of many of them have been defined in HapMap, which encompasses a set of four world populations, and in the Human Genome Diversity Panel, a collection of 51 smaller population samples worldwide. However, despite efforts such as the ENCODE Project, our ability to identify variations in the human genome has vastly exceeded our understanding of their biological significance, a problem that is particularly relevant in attempts to unravel the origins of common disorders such as drug addiction.

In this review, we summarize the current knowledge of genes implicated in the etiology of addictions (**Figure 1**). We explore the future of genetic research in this field and the challenges that need to be addressed, such as a lack of replication of association studies, the somewhat disappointing results from genome-wide association (GWA) studies conducted to date, and the importance of identifying functional predictors to help make sense of the information gained. Furthermore, gene–environment (G × E) interactions have not been studied extensively in addiction despite their potential importance. Several examples of genetic testing in medicine are well known; we examine how genetic findings might be translated into clinical practice for the prevention and treatment of addictions. The need for and importance of pharmacogenetic studies of functional variants in the understanding of disease and therapeutics are discussed. The use of genetic information presents a variety of ethical and practical challenges, several of which are either unique to or especially salient in drug addiction. A comprehensive list of relevant publications related to the sections discussed is provided in **Supplementary Table S1** online.

GENES AND ADDICTION: WHAT DO WE KNOW?

A common feature of all drugs with potential to cause addiction is their ability to activate the mesolimbic brain reward pathway and increase dopamine levels in the nucleus accumbens.[8] This can occur via facilitation of dopamine release from presynaptic neurons or by inhibition of its reuptake (e.g., the effects of cocaine and amphetamines) or by increasing the activity of dopaminergic neurons (e.g., the effects of alcohol, nicotine, opioids, and cannabis).[8] Although dopamine plays an important role in mediating the rewarding properties of drugs of abuse,

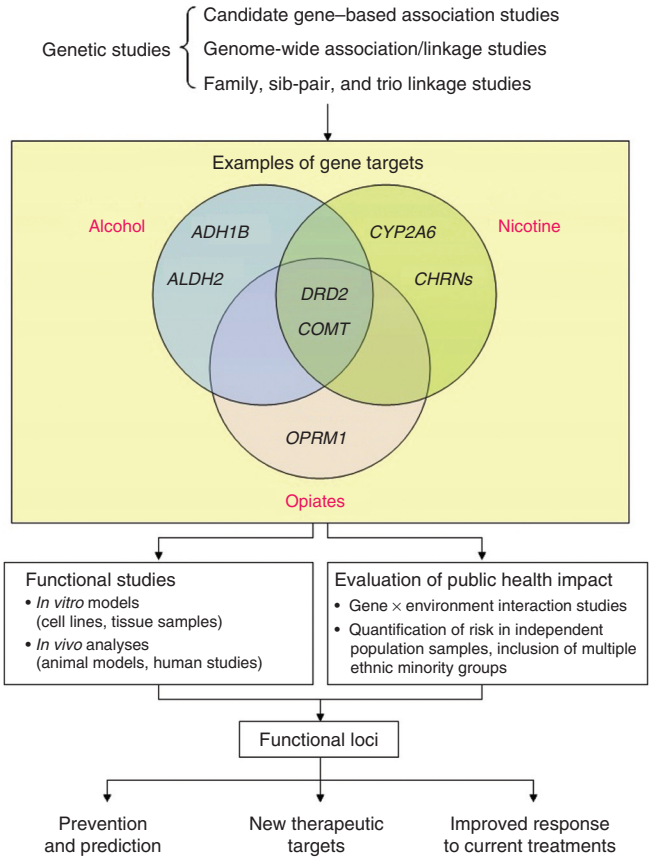

Figure 1 Identification of genes underlying drug addiction and applications of gene-related information. Genetic loci for drug addiction have been discovered through candidate gene–based association studies, genome-wide association/linkage studies, and family-based linkage studies. Some of the genes, such as those in the brain reward pathways, are involved in a variety of drug addictions, whereas others are more drug specific. The functional characterization of these genetic loci, in conjunction with evaluation of the associated public health impacts, will help to identify new targets with utility in the prevention and treatment for drug addictions.

other neurotransmitters, including serotonin, opioid peptides, γ-aminobutyric acid, acetylcholine, endocannabinoids, and glutamate, also contribute.[8] It is notable that learning processes are crucial to the neurobiology of addiction. In both humans and laboratory animals with addiction, drug-associated conditioned stimuli can evoke dopamine release alone.

In general, genes implicated in addiction can be categorized into those that influence the likelihood of initial experimentation with addictive drugs, or those that are directly involved in the biological processes underlying addiction once the individual has been exposed to the drug. Therefore, genes that are related to personality traits (such as impulsivity, risk taking, and response to stress) may predispose an individual to experiment with drug use, whereas others may be involved in the initial, immediate, subjective, and physiological reaction to drug use, helping to determine whether the drug use will continue and escalate. Genes that encode proteins involved in the brain reward system are also important in the development of dependence to various classes of drugs. Variability in the function of receptors, transporters, and metabolic enzymes of the aforementioned neurotransmitter systems may modify the risk of drug dependence. For example, variability in the gene(s) encoding the dopamine D_2 receptor (*DRD2*) and/or its adjacent ankyrin repeat and protein kinase domain–containing protein 1 (*ANKK1*) has been implicated in addiction to nicotine, heroin, and cocaine, as well as to alcoholism and abuse of psychostimulants.[7,9] In addition, genes that influence dependence in a drug-specific manner have also been implicated. For example, variability in cytochrome P450 2A6 (*CYP2A6*), the main enzyme involved in the metabolism of nicotine, has been associated with different kinds of smoking behavior.[4] **Table 2** summarizes the various candidate genes implicated in drug addiction and includes citations for more detailed reviews.

In addition to using more traditional genotyping approaches, research on addiction and other complex diseases have been utilizing GWA studies during the past few years; large numbers of single-nucleotide polymorphisms (SNPs) (ranging from several hundred thousands to more than one million) spread across the genome are assessed in affected individuals and controls, and the associations with disease outcome are analyzed. SNPs that are in high linkage disequilibrium (i.e., SNPs that tend to be inherited together more commonly than would be expected by mere chance) can serve as proxies for each other. Therefore, not all the markers within a given region need to be genotyped, as long as the marker panels can capture the variations at the loci that have not been genotyped.[10] GWA studies have provided new insights into our understanding of the genetics of addiction because they are conducted without *a priori* hypothesis based on gene function or disease pathways as described above. As a result, a number of novel targets with potential biological relevance have been discovered. For instance, GWA studies have found associations between genes involved in cell adhesion such as neurexin 1 (*NRXN1*) and nicotine dependence, and neurexin 3 (*NRXN3*) has been implicated in alcohol, opioid, and polysubstance abuse (reviewed in ref. 7; **Supplementary Table S1** online). Furthermore, the association between the *CHRNA5-A3-B4* gene cluster and smoking-related illnesses such as lung cancer and chronic obstructive pulmonary disease was initially revealed by GWA studies that reported significant associations between the occurrence of these diseases and the chromosomal regions 15q24–25 (reviewed in ref. 11; **Supplementary Table S1** online). These novel targets provide clues to the neurobiology of addiction but require more detailed assessment of their functionality, as discussed below.

On a parallel track, as our understanding of the neurobiology of addiction improves, we have novel candidate genetic targets to consider. One example is that of microRNAs that interact with the 3′-untranslated region (3′-UTR) of the target mRNA and can mediate their degradation or repress translation. With the development of drug dependence, the brain undergoes significant remodeling and adaptation, and the expression profiles of a number of genes in the brain are known to change following acute and repeated administration of psychostimulants and other drugs of abuse.[12] These changes are thought to represent a compensatory mechanism to maintain homeostasis

Table 2 Selected examples of genetic variations that have been implicated in drug addiction phenotypes

Target	Gene(s)	Phenotype
Brain reward pathways		
Dopamine		
Receptors	*DRD2*	Smoking initiation, persistence, cigarette consumption, cessation Heroin use, cocaine dependence Psychostimulant polysubstance abuse Alcoholism
	DRD4	Smoking risk, time to first cigarette, craving and response to smoking cues, nicotine dependence Heroin, cocaine dependence Methamphetamine use
Transporters	*SLC6A3 (DAT1)*	Smoking risk Cocaine dependence
Metabolism		
Monoamine oxidase A	*MAO-A*	Cigarette consumption, smoking risk, nicotine dependence
Tyrosine hydroxylase	*TH*	Smoking risk
Dopamine β-hydroxylase	*DBH*	Smoking risk, nicotine dependence
Catechol-*O*-methyltransferase	*COMT*	Smoking risk, treatment response to nicotine spray and patch, nicotine dependence Heroin, cocaine dependence Alcohol dependence Methamphetamine use
Serotonin		
Transporters	*SLC6A4 (5HTT, SERT)*	Smoking risk, cigarette consumption, dependence Alcohol dependence Heroin dependence
Metabolism		
Tryptophan hydroxylase	*TPH1, 2*	Age of smoking initiation, risk of smoking Heroin dependence Alcohol dependence
Pharmacodynamic targets		
Opioid receptor (μ, δ, κ)	*OPRM1*	Heroin, opiate dependence Alcohol dependence
	OPRK1	Heroin, opiate dependence Alcohol dependence
	OPRD1	Heroin, cocaine dependence
Nicotinic acetylcholine receptor, α_4 subunit	*CHRNA4*	Nicotine dependence
Nicotinic acetylcholine receptor, α_5, α_3, β_4 subunits	*CHRNA5, A3, B4 gene cluster on chr. 15*	Nicotine dependence, cigarette consumption, lung cancer, risk of chronic obstructive pulmonary disease Alcohol, cocaine dependence
Cannabinoid receptor		
$GABA_A$ receptor, γ2 subunit	*GABRG2*	Heroin dependence, methamphetamine use
$GABA_A$ receptor, α_2 subunit	*GABRA2*	Alcohol dependence
$GABA_B$ receptor, subunit 2	*GABBR2*	Nicotine dependence
Pharmacokinetic targets		
Cytochrome P450 2A6	*CYP2A6*	Smoking risk, cigarette consumption, smoking cessation
Alcohol dehydrogenase-2	*ADH2, ADH4, ADH1B, ADH1C*	Alcohol dependence
Aldehyde dehydrogenase-2	*ALDH2*	Alcohol dependence
Cytochrome P450 2E1	*CYP2E1*	Alcohol dependence

For a comprehensive list of primary references, see **Supplementary Table S3** online.

in response to drug-induced effects and are manifested as drug-seeking behaviors, withdrawal, and tendency to relapse. With the discovery of microRNAs and their role in the regulation of gene expression, it has been proposed that drug-induced gene expression changes may be mediated through this intermediate pathway. One study has shown that nicotine treatment up- and downregulates a number of microRNAs, in particular miR-140*, which regulates the expression of a number of genes including

dynamin 1 (*Dmn1*). This gene may be involved in endocytosis and is possibly important in drug-induced neural plasticity[13] and nicotine dependence.[14] Similarly, miR-504* has been implicated in the regulation of allele-specific differential expression of a functional SNP in the 3′UTR of *DRD1* that has been associated with nicotine addiction.[15]

Recent evidence also suggests that epigenetic regulation of gene expression may contribute to the pathogenesis of drug addiction. In the nucleus accumbens of rodents, cocaine increased histone acetylation of promoters in a number of genes (*cfos*, *fos-b, bdnf, cdk5*, and *npy*) that are known to be important in the addiction process (reviewed in ref. 16; **Supplementary Table S1** online). Also, higher methylation levels in the promoter region of *OPRM1* have been found in the lymphocytes of former long-term heroin addicts undergoing methadone maintenance treatment.[17] Alcohol withdrawal increased the expression of histone deacetylases and decreased the expression of CREB and NPY in the amygdala in rodent models, whereas inhibition of histone deacetylases reduced the anxiety resulting from alcohol withdrawal.[18] Therefore, variability in these gene regulators may be additional important determinants of drug addiction phenotypes.

GENES AND ADDICTION: WHERE DO WE GO FROM HERE?

Need for better replication of results

Despite enormous efforts over the past few decades, the progress in finding the genes and causal variants underlying drug addiction has been slow. The variants examined in candidate gene association studies so far have been based on a rather imperfect understanding of biological pathways, and studies have often yielded inconsistent results. For example, although case–control association studies have associated the *Taq1* A1 allele with a number of smoking and alcoholic phenotypes, several other studies have reported negative results, and meta-analyses have generally failed to support an association (**Table 2**; **Supplementary Table S3** online).

Similarly, given that GWA studies necessarily involve multiple statistical tests, stringent levels of significance are required, thereby hindering the replication of results. At a nominal *P* value of 0.05, a GWA study examining 500,000 SNPs could potentially result in 25,000 false positives. For this reason, a genome-wide statistical significance of $P < 1 \times 10^{-7}$ is typically used; however, this calls for very large sample sizes so as to ensure sufficient statistical power. Therefore, in order to address the potentially high number of false-positive results, it is particularly vital to replicate early results in independent samples; this is now often included in the same GWA study as part of a multi-stage design. However, replication of initial findings demonstrating similar magnitude and direction of effect within the same or similar phenotype and population is often not observed, thereby complicating the interpretation of results.[10] It is notable, however, that some of the associations can be quite robust and replicable, such as the association of the *CHRNA5-A3-B4* gene cluster with smoking phenotypes.[11]

There are several possible explanations for the lack of replication. Addiction is a complex behavioral trait, and substantial heterogeneity exists between studies because there are a number of variables that may differ or may not have been controlled for across studies. Many studies have been underpowered, and different study methodologies (e.g., prospective vs. retrospective and population-based vs. clinical trial samples) may result in different sample populations. There is also a lack of consistency in phenotypes and outcome measures, such as definition of an appropriate control group (e.g., ever vs. former vs. never users). Inclusion of individuals of different ethnic backgrounds, although important for understanding predictors of diseases in these populations, may result in erroneous conclusions due to population stratification, given that frequencies of alleles and cultural acceptability of drug use may differ across populations. GWA studies have indicated potential population stratification even within geographical regions previously considered to be genetically homogeneous. As such, the use of better selected controls and examination of ancestry-informative markers may address these issues to an extent. The use of intermediate phenotypes, as discussed below, may also help improve reproducibility of studies by reducing the heterogeneity among studies. Publication bias, in which positive results are more likely to be published, may inflate the apparent effect of a genetic variant and its association to addiction outcomes. Care must also be taken to ensure that claims of replication of data are made with sound justification, so as to avoid further confusion in the literature. These issues occur widely in genetic research, including in studies of drug addiction, and need to be considered in the design of future genetic studies.

Need for more consistent definitions of phenotypes

Two of the biggest challenges of genetic research in drug addiction are the heterogeneity of the phenotypes studied and the lack of consistent measurements for outcomes across studies. For instance, persons with alcoholism vary greatly with respect to the age of onset of problem drinking, alcohol symptoms, drinking history, and comorbid disorders. There are a number of scales to measure nicotine dependence; the two most commonly used are the Fagerström Test for Nicotine Dependence (FTND) and the criteria in the fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV). However, these two measures correlate only weakly,[19] and they probably capture different aspects of dependence. The FTND may be a stronger measure of physical dependence, whereas the DSM-IV emphasizes the awareness of dependence, such as recognition of adverse consequences of smoking, a desire to reduce use, and mood changes that occur during withdrawal. Consequently, there is a need for consistent instrumentation so that data from different studies can be combined and/or compared and for the development of measures that better capture the multidimensional nature of dependence and its evolution over time in the individual's addiction history. Limiting the use of retrospective, self-report data may also help reduce inconsistencies in phenotype outcomes; biomarkers should be incorporated whenever possible to confirm the self-reports from the individuals with addictions. In addition, proxy (e.g., sibling and spouse) reports can be used to provide supporting information, given

that medical records rarely contain a comprehensive record of substance use and not all drug-dependent subjects seek treatment. Funding agencies and scientific societies should make a major effort to ensure that all large-scale studies in drug addiction incorporate a core set of measures that are fully comparable, starting with definitions of use, quantity, and frequency of intake, as well as aspects of abuse and dependence.

In recent years, intermediate phenotypes have been proposed as an alternative to traditional phenotype measures. These include neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, and neuropsychological correlates that are heritable and quantifiable and are thought to more closely manifest etiologies of addictions. The relationship between genes and clinically observable symptomologies of addiction is highly complex, and intermediate phenotypes are thought to capture information on mediating variables within this chain of events. Because they are objective measures and are potentially less complex than the diagnostic criteria for addiction that involve wide-ranging symptomologies and clinical courses and associations with environmental exposures, intermediate phenotypes may be more representative of gene action. Electrophysiological measures, as well as structural and functional imaging data in the so-called imaging genetics studies, have been used to investigate brain alterations associated with alcohol dependence in relation to genetic variations in *GABRA2* and *CHRM2* (reviewed in ref. 20; **Supplementary Table S1** online), *COMT* and *mGluR3* (ref. 21), and *DRD4* and *OPRM1* (ref. 22).

Where is the missing heritability?

When GWA studies were first introduced in the field of genetic research of complex diseases, their premise and method were simple: screen for association using markers spaced evenly across the genome in order to capture the effects of most, if not all, the common genetic variations in any individual, determine their associations with phenotypes of interest, and assess these loci for their functional effects in relation to the disease process. However, some major challenges rapidly arose. The commonly occurring alleles examined in these studies account for relatively small increments in risk and explain only a minor proportion of the phenotypic variance observed, with the odds ratios of associated SNPs being in the range of 1.1–2.0 (ref. 23). Much of the genetic variance for a number of complex traits has not been accounted for. Height in humans is one example; the estimated heritability of height is ~80%, but only ~6% of the phenotypic variance is accounted for by GWA studies.[23] A similar observation was made for nicotine addiction; the proportion of variance in cigarette consumption accounted for by markers identified in GWA studies ranges from only ~1% (relating to cigarettes per day) to ~4% (relating to cotinine levels),[24] even though the heritability of this trait has been estimated to be ~50%.

The failure of GWA studies to account for this apparent missing heritability has stimulated debate on the utility of these studies, particularly given the enormous financial and scientific investments dedicated to them. The relative merit of this approach has been debated and discussed in greater detail elsewhere.[10,23] The common disease/common variant hypothesis, which states that common complex diseases are attributable to relatively few common genetic variants of moderate effect, has not been supported. Instead, the genetic underpinning of addictions and other complex diseases may be attributed to either multiple common variants, each contributing a very minor role, or multiple rare variants with intermediate to larger effect sizes, for which the resulting odds ratios fail to reach genome-wide statistical significance with the attainable sample sizes. In the past few years, GWA studies have been performed on a very large scale, with tens of thousands of samples from multiple independent studies, in order to identify the effects of common variants with small effect sizes. However, caution needs to be exercised to ensure that these large sample sets are not collected at the expense of detailed environmental and behavioral information, that they do not limit the use of intermediate phenotypes, and that internal heterogeneity within studies does not offset the advantage of increased sample size. These meta-analytic studies are designed to detect the residual main genotype effects and probably to identify only those variants whose main effects are large enough to be detectable against the backdrop of the myriad different environments from which these samples are taken.

Targeted resequencing of genes that have previously been found to have common variants associated with complex diseases, or of functionally related genes, may be another method by which new sources of variation can be discovered. For example, four rare genetic variants (each with an allele frequency of ~1%) were found through resequencing; these taken together accounted for a greater proportion of the variance in the risk of developing type 1 diabetes than a single common variant located in the same gene as detected by GWA studies.[25] In addition, whereas SNPs represent alterations at single nucleotides, which may only reduce function to a modest extent, other larger types of structural changes to genetic architecture, such as copy number variations, may have larger effects on gene function and consequently a greater phenotypic impact. The 1000 Genomes Project (http://www.1000genomes.org), an international consortium with the goal of creating a complete, detailed catalog of all genetic variations (including rare SNPs, copy number variations, and insertions/deletions) in at least 1,000 genomes from across the world, will serve as a useful reference for future studies of addictive disorders. It is notable that, although samples will be drawn from various world populations, some populations will probably be underrepresented, and therefore detection of variants present in these groups may be missed.

Discovery of functional predictors

Another limitation of GWA studies is that the markers identified are often not the causal variants themselves but rather are in linkage disequilibrium with them. This may contribute to the lack of reproducibility between studies because these markers may be in linkage disequilibrium in one population but not in another. Furthermore, causal variants may not be well tagged by SNPs used in commercial genotyping arrays. This highlights the

importance of identifying *functional* genetic predictors involved in complex diseases such as drug addiction.

Genetic manipulations in cell or animal models, as well as studies using tissue samples or cell lines, can be used to elucidate the functional impact of genetic variants. One example is the case of the *CHRNA5-A3-B4* cluster on chromosome 15, encoding nicotinic acetylcholine receptor (nAChRs) subunits, which has been implicated as a susceptibility locus for nicotine addiction and lung cancer in numerous GWA studies. Even though a number of variants within the *CHRNA5-A3-B4* cluster have been implicated in nicotine addiction, a consensus has not yet been reached on the mechanism through which the receptor subunits may be mediating these phenotypes. For example, it is not known whether these genes are involved in initiation, progression, or maintenance of nicotine dependence, although there is growing consensus that the genes are associated with the extent/heaviness of the smoking habit. Furthermore, whether the association of this gene cluster with lung cancer and chronic obstructive pulmonary disease is a direct and/or indirect effect through an influence on the extent of cigarette consumption remains to be clarified. Understanding the biological significance of the *CHRNA5-A3-B4* receptor subunits and the functional significance of the variants implicated in this gene cluster will help interpret the observed associations.

The nAChRs are pentameric ligand–gated cation channels consisting of some combination of nine α (α_2–α_{10}) and three β (β_2–β_4) subunits. Mouse knockout models have demonstrated that the α_4 and β_2 subunits, the most widely expressed forms in the brain, are critically important for nicotine self-administration and nicotine-induced dopamine release in the ventral tegmental area.[26,27] Mouse knockout models for α_5, α_3, and β_4 subunits have also been created (although only in heterozygous form for α_3 because the complete knockouts suffer severe physical abnormalities and die within weeks of birth).[28] Their responses to nicotine have not been extensively analyzed yet, although in general, these animals have reduced sensitivity to nicotine-induced seizures and the locomotor suppressant effects of nicotine (reviewed in ref. 29; **Supplementary Table S1** online). Animal models will also be useful in determining whether these nAChR subunits have a direct role in mediating lung cancer or chronic obstructive pulmonary disease, apart from their effect of altering smoking behaviors.

Functional tests have also shown that rs16969968, a nonsynonymous SNP (Asp398Asn) in *CHRNA5* implicated in GWA studies, lies in the M3–M4 intracellular loop of the receptor and may be involved in receptor trafficking but not receptor expression levels.[30] Although such study reports are encouraging, the functional significance of genetic variations in the *CHRNA5-A3-B4* gene cluster is not yet clear.

It is also notable that SNPs that have been implicated in this region also lie within genes encoding an iron regulatory protein (*IREB2*), an α_4 proteosome subunit protein (*PSMA4*), and a putative protein of unknown function (LOC123688) that may also be important in cell proliferation and apoptosis.[11] Further research, such as behavioral tests in animal models and targeted resequencing of this gene cluster, will help clarify the role of these genes and their variants in mediating smoking phenotypes. This also provides the basis for the hypothesis-driven testing of gene–gene interactions, for example, by conditioning analyses on a known functional SNP.

G × E interactions

The gene-related influence on any behavioral outcome is likely to depend on exposure to certain environments; for example, regardless of genetic propensity for addiction, an individual cannot become addicted to a drug if he or she is never given the opportunity to experiment with it. It is becoming clear that research on addiction should not be restricted to a study of either environmental or genetic effects in isolation but rather on G × E interactions, including the tendency of people to nonrandomly assort to particular environments (G–E correlation). However, the majority of studies related to drug addiction do not account for G × E interactions, although a few studies in alcoholism report having taken the interaction into consideration. Several studies have reported interactions of *5-HTTLPR, MAO-A-LPR*, and *DRD2 TaqA1* genotypes with family relationships, maltreatment, or negative life events as influencing alcohol use, intoxication, and dependence (reviewed in ref. 31; **Supplementary Table S1** online). Additional instances in which the influence of genetic variation on function may depend on G × E interactions have been studied. The association between high 5-HTT expression and low levels of response to alcohol resulting in higher intake of alcohol has been reported in individuals with two copies of the long allele of *5-HTTLPR*; a similar effect was also observed among carriers of the short allele who had been exposed to early developmental stress.[32] These findings demonstrate that the presence of G × E interactions may potentially confound the interpretation of data from genetic association studies, account for part of the "missing heritability," and contribute to the lack of reproducibility between studies. However, in the majority of cases, the main genotype effect should still be observed regardless of environmental influences, if the study is sufficiently powered.

A number of challenges underlie G × E studies, and their application remains controversial to some. One of the main issues is that, just as the genetic factors underlying drug addiction are not well known, the set of relevant environmental factors is also not clearly understood. Therefore, the discovery of G × E interactions depends on having appropriate subgroups (particularly those with a sound theoretical or biological basis) in the analyses wherein the genetic factors are expected to have an effect. Inappropriate subgrouping may lead to erroneous conclusions of a lack of genetic, environmental, or G × E effects, and failure to replicate a G × E result in a subsequent study may be due to differences in the two studies' subgroups. However, particular care must be taken in selecting subgroups because unguided exploration through the endless permutations of possible subpopulations increases the risk of type 1 errors, making the criteria for replication more stringent. Well-validated and consistent measurements of environmental factors are needed because some G × E interactions have been shown to be artifacts caused by scaling of environmental

measures. The lack of a clear distinction between environmental and genetic influences is also an issue. For example, bad parenting as an adverse environmental risk factor is in part related to genetics as manifested by individual differences in personality traits. Other contextual variables, such as social cohesion in the local community, may also be needed in the analysis. For example, certain G × E interactions (e.g., gene–parenting interactions) may appear in certain cultures but not in others because of differences in the contexts of drug use and abuse. Multilevel analyses may be one approach to modeling these effects. Finally, the relative importance of genetic and environmental factors, as well as their interactions, will vary according to the life stage of the individual. Therefore, longitudinal and age-specific models may be important, particularly for drug addiction.

It is notable that, despite the potential importance of G × E interactions, only some of the studies dealing with complex diseases have attempted to take these effects into account. Although methodological issues in the design and analysis of gene–environment effects remain, some methods have recently been proposed to account for these interactions in GWA studies.[33] Furthermore, the inclusion of environmental covariates into the analyses of data generated by GWA studies revealed associations with new genetic loci, and their levels of statistical significance were higher as compared with the results of unadjusted analyses.[34] This shows that a due consideration of genetic and environmental factors and their interactions may enhance the ability to detect true risk factors.

Re-emergence of family linkage studies

Family linkage studies examine genetically related individuals exposed to a similar familial—and, to some extent, community—environment; these studies have been of great utility in genetic epidemiology and have proven effective in identifying genes responsible for simple Mendelian diseases such as cystic fibrosis. Related individuals affected with the disease are recruited along with their unaffected family members, and the inheritance pattern of the disease is examined through genomic markers. Differential transmission of alleles to the affected individuals indicates linkage of the marker with the phenotype measured. However, obtaining samples for family-based studies, particularly for psychiatric illnesses such as drug addiction, can be difficult given the associated stigma. Genotyping costs are also greater because of the higher density of markers required as compared with studies with population-based samples, and the large size of chromosomal regions shared between family members makes it more difficult to isolate the signal associated with disease outcomes. Nevertheless, in comparison with GWA studies, which use population-based samples and are powered to detect common variants with modest effects, traditional family linkage studies may have greater power to detect rare variants associated with drug addiction phenotypes. To some extent, the same argument applies to the use of founder populations and genetic isolates in which some variants that are rare on a worldwide basis can be expected to be far more common in these samples.

HOW CAN GENETIC INFORMATION BE APPLIED TO DRUG ADDICTION?

Significant pharmacogenetic impacts on drug addiction

In several studies, functional genetic variants were found to have a clinically relevant pharmacogenetic impact on drug addiction. Only a brief summary of these important examples is provided here as they have been reviewed more extensively elsewhere.[35–38]

The discovery of functional polymorphisms in alcohol dehydrogenase IB (*ADH1B*) and aldehyde dehydrogenase-2 (*ALDH2*) represents one of the earliest and still most successful examples of pharmacogenetics as applied to drug addiction. These enzymes catalyze consecutive steps in alcohol metabolism; both have functional polymorphisms common in East Asians that additively alter the risk of alcoholism, with protective effects of 4- to 10-fold, depending on the population.[35] The two most common genetic variants in these enzymes are *ADH1B* His47Arg, in which Arg47 is an increase-of-function allele, and *ALDH2* Glu487Lys, in which Lys487 is an inactive allele. Accumulation of acetaldehyde, the intermediate from ADH metabolism, potently releases histamine, triggering skin flushing, headaches, nausea, and palpitations that are thought to deter heavy alcohol use and development of dependence. Indeed, several studies have shown that genetic polymorphisms in *ADH1B* and *ALDH2* that have functional impacts on enzyme function alter the risk of alcohol dependence (reviewed in ref. 9; **Supplementary Tables S1** and **S3** online). Acetaldehyde is also a mutagen that can react with a variety of biomolecules; therefore, individuals with the Glu487Lys loss-of-function variant in *ALDH2* should refrain from drinking large quantities of alcohol because they face a substantially elevated risk of upper gastrointestinal cancer as compared with individuals with the fully active enzyme.[36]

Similarly, genetic variants in the μ-opioid receptor have significant implications in drug addiction. A SNP (118A>G) that corresponds to an amino acid change of Asn40Asp in the N-terminus of the receptor is of particular interest. Receptors with this variant have a threefold greater binding of beta-endorphin, with a corresponding threefold greater activation of the G-protein coupled inwardly rectifying potassium channels, although no differences in binding or activation by other endogenous opioid peptides or exogenous opiates were observed.[39] In specific cell lines, morphine, methadone, and the synthetic opioid peptide DAMGO are all less potent at inhibiting adenylyl cyclase activity for receptors with the Asp40 variant. The Asp40 SNP also lowers receptor levels in expression cell systems as compared with the wild-type Asn40 allele, possibly because of differences in glycosylation.[39] The presence of this variant influences the diverse physiological functions under modulation by the μ-opioid receptor, such as stress responsivity and pain perception. Processes related to abnormal stress responsivity, such as alcohol and opioid addictions, were found to be associated with genetic variations at the μ-opioid receptor, even though these are distinctly different disorders (reviewed in ref. 38; **Supplementary Tables S1** and **S3** online). Several studies, including those in the relatively non-admixed populations of central Sweden and in populations of Han Chinese, have shown that the Asn40Asp variant is

associated with opiate addiction. One study has shown that it is also associated with alcoholism, although other studies have reported negative results[38] (**Supplementary Tables S1** and **S3** online). Of particular pharmacogenetic and clinical significance is the fact that the presence of one or two copies of this variant predicts a favorable outcome to treatment of alcoholism with naltrexone, a selective opioid antagonist.[40]

It has been demonstrated that smokers titrate the levels of their smoking in order to maintain a particular level of nicotine in their systems and that manipulation of the rates of nicotine clearance alter smoking behaviors (reviewed in ref. 37). It follows that variability in CYP2A6, the main metabolic inactivating enzyme for nicotine, can influence smoking behavior, dependence, and cessation. Currently, 38 *CYP2A6* alleles have been identified, including SNPs, gene conversions, deletions, and duplications (http://www.cypalleles.ki.se/cyp2a6.htm), and much progress has been made in understanding their functional impact. Many of these genetic variants significantly alter the rate of nicotine metabolism in a variety of populations (reviewed in ref. 37; **Supplementary Table S1** online). Consistent with this finding, many studies (primarily of heavy-smoking individuals of Caucasian or Japanese ethnicity) have shown that smokers with genetic variants that impair CYP2A6 function, thereby reducing nicotine metabolism, smoke fewer cigarettes, are less likely to be adult current smokers, and have a lower risk of lung cancer (reviewed in refs. 4,37; **Supplementary Tables S1** and **S3** online). Some studies have also indicated that reduced CYP2A6 activity may also alter the rate of acquisition of nicotine dependence and the rate of escalation of dependence.[41,42]

Genetic testing in the prevention of drug addiction

Genetic testing already has important clinical applications in the prevention of diseases. Screening for deleterious variants in *BRCA1/2* associated with increased risk of hereditary breast and ovarian cancer in women is becoming more common, and individuals with deleterious variants of *BRCA1/2* can increase surveillance (e.g., by increasing the frequency of mammograms), undergo prophylactic surgery, and take steps to reduce other risk factors. However, implementation of genetic testing in prevention programs for drug addiction has additional complexity because experimentation and initiation of drug use is a matter of choice made by the individual. It is unclear whether prevention programs based on certain genetic vulnerabilities to developing drug dependence, targeted in particular to youths and adolescents who are the prime targets of such interventions, will be effective or socially acceptable. Although we are still far from having sufficiently powerful genetic predictors for addictions, one example of a genetic predictor that could be useful even today is the Glu487Lys variant of aldehyde dehydrogenase-2 (*ALDH2*), which is found in approximately 500 million people. Physicians should inform patients carrying the loss-of-function variant that they are at elevated risk of upper gastrointestinal cancer and suggest that they refrain from consuming large amounts of alcohol.[36]

Legislation to control accessibility and education to change the acceptability of drugs of abuse have already shown great potential to reduce rates of use among adolescents, particularly with respect to smoking.[43] Although it may be difficult to incorporate genetic testing into drug prevention programs, genetic research related to drug addictions can assist in understanding the mechanisms underlying the etiologies of such addictions. The prospects of using genetics to tailor medical treatment for drug addiction are encouraging.

Genetic testing in the treatment of drug addiction

Once an individual is addicted to a drug, the currently available clinical options for treatment are rather limited and only partially effective. Current pharmacotherapies and behavioral counseling improve the likelihood of smoking cessation by ~1.5- to 2.0-fold, with high rates of relapse and an average of 4–5 quit attempts needed before success. Personalized medication choices can be facilitated to a great extent by genetic studies, which provide novel insights into the pharmacodynamics, pharmacokinetics, and other aspects of the disposition of medications. Pharmacogenetic studies have already shown the ability to use genetic testing to identify individuals who respond better to certain types of therapies for drug addiction (**Table 3**). For example, functional variations in *DBH, DRD2, OPRM1, CYP2A6*, and *CYP2B6* have been associated with smoking abstinence rates in clinical trials, in response to either pharmacotherapy or placebo, although not always consistently (reviewed in ref. 4; **Supplementary Table S4** online). Other pharmacogenetic studies have examined treatment response for alcohol and opiate dependence. Variability in genes that are involved in methadone metabolism, such as *CYP3A4* and *CYP2D6*, as well as genetic variations in P-glycoprotein (*ABCB1, MDR1*), for which methadone is a substrate, and *DRD2* may alter the methadone dosage required for maintenance in heroin addicts.[44–46] However, methadone is a complex drug for which the pharmacokinetic and pharmacodynamic responses are not well understood, and the genetic factors that underlie the variability in the response to methadone therapy are not well known.

A few genetic tests to optimize medical treatments in psychiatry, either by enhancing therapeutic efficacy or reducing adverse effects, are already in place. The US Food and Drug Administration has added the requirement for genetic testing to the prescription of carbamazepine in the treatment of bipolar disorder and neuropathic pain, because variations in the human leukocyte antigen gene (*HLA-B*1502*) have been associated with a potentially fatal skin reaction (reviewed in ref. 46). The agency also approved the first diagnostic pharmacogenetic test in 2005. The AmpliChip CYP450 genotyping platform assesses variants in *CYP2D6* and *CYP2C19*, the enzymes that metabolize numerous drugs including antidepressants, antipsychotics, and opiates. It is intended to help physicians prescribe the type and dosage of medications on the basis of an individual's genotype.[46] The next step is to carry out prospective studies to determine whether pharmacogenetic testing can improve treatment outcomes for drug dependence and to determine whether such procedures are cost-effective as compared with standard care. Such economic analyses have already been performed for smoking cessation treatments, and the results suggest that

Table 3 Common pharmacotherapies for drug dependence and the genetic variations that have been implicated in treatment response

Treatment	Mechanism of action	Gene(s)	Effect
Nicotine dependence			
Nicotine replacement therapy (gum, transdermal patch, lozenges, nasal spray)	nAChR agonist	*CYP2A6*	*CYP2A6* slow metabolizers, as indicated by phenotype indicator (*trans*-3′hydroxycotinine/cotinine ratio), were found to have higher quit rates
		DRD2/ANKK1	*Taq*1A A1 allele is associated with higher quit rates as compared with the A2 allele *DRD2*-141C Del genotype was associated with higher quit rates as compared with *DRD2*–141C Ins genotype *DRD2* 957T allele was associated with higher quit rates compared with the C allele
		DBH	*DBH* 1368A allele was associated with higher quit rates as compared with the G allele
		COMT	For the *COMT* Val108/158Met polymorphism, individuals with the low-activity Met allele had higher quit rates
		OPRM1	For the Asn40Asp polymorphism, the Asp40 variant was associated with higher quit rates as compared with those homozygous for Asn40
Bupropion	Dopamine/norepinephrine reuptake inhibitor Noncompetitive inhibitor of nAChR	*CYP2B6*	Effect primarily driven by low quit rates of *CY2B6*6* in placebo arm
		DRD2/ANKK1	*Taq*1A A2 allele was associated with higher quit rates Individuals homozygous for the *DRD2 -141C Ins* allele had significantly higher quit rates
		CHRNB2	GG genotype for 3′UTR SNP (rs2072661) was associated with higher quit rates
Varenicline	$\alpha_4\beta_2$ nAChR partial agonist	None examined to date	
Alcohol dependence			
Disulfiram	Aldehyde dehydrogenase inhibitor Dopamine β-hydroxylase inhibitor	None examined to date	
Naltrexone	Nonselective opioid receptor antagonist	*OPRM1*	Individuals with the Asp40 variant had significantly lower rates of relapse and longer time to return to heavy drinking as compared with those homozygous for Asn40, although no differences were seen in overall abstinence rates
Acamprosate	Unclear, may be metabotropic glutamate receptor subtype 5 (mGluR5) antagonist	None examined to date	
Opiate dependence			
Methadone	Opiate receptor agonist	*ABCB1* (MDR1) encoding P-glycoprotein	Those with TT genotype for rs1128503 C>T SNP were more likely to receive higher methadone doses
		DRD2/ANKK1	Individuals with the T allele of *DRD2* rs6275C>T SNP required higher doses of methadone for maintenance therapy
Levo-α-acetylmethadol	μ-Opioid receptor agonist	None examined to date	
Buprenorphine	μ-Opioid receptor partial agonist κ-Opioid receptor antagonist	None examined to date	
Naltrexone	Nonselective opioid receptor antagonist	None examined to date	

SNP, single-nucleotide polymorphism.

Reviewed in refs. 4,9,44. A complete list of references can be found in **Supplementary Table S4** online.

genetic testing can be beneficial under certain assumptions.[47,48] These include the allele frequency of the genetic variant examined being neither too common nor too rare and the treatment response effect size of one genotype group being sufficiently larger than the other. Even though genetic testing may not necessarily be more cost-efficient in some cases,[48] a demonstration that individuals with certain genetic variants will respond better to this treatment may encourage its use among those who would otherwise be reluctant to use it. There is already evidence that feedback to a patient about the results of genetic testing can result in behavioral modifications related to drug addiction. For example, Marteau and Munafò *et al*. (personal communications) have also demonstrated that disclosure of genetic information can alter behavior and treatment compliance for smoking cessation.

CHALLENGES AND BARRIERS TO GENETIC RESEARCH IN DRUG ADDICTION

Informatics

A plethora of data can now be generated through GWA studies, RNA expression studies based on microarrays and RNA sequencing, DNA sequencing and methylation studies, studies of epigenetic changes in histones, and resequencing studies targeting both SNPs and copy number variations. However, many research laboratories are understaffed and ill-equipped to face the informatics technology challenges inherent in these high-throughput methodologies. Also, research is constrained by software and hardware capabilities to handle extensive clinical genetic databases. It should be noted that these issues are applicable to all genetic studies of complex diseases and have been covered in greater detail elsewhere; a brief discussion is provided here.

The informatics technology challenge exists at several levels: (i) planning (e.g., the informatics required to generate DNA capture arrays for sequencing); (ii) data capture, transfer, processing, and storage (data files can be as large as five terabytes); (iii) primary analyses, including mapping of hundreds of millions of DNA fragments to reference genomes; (iv) secondary analyses, including identification of SNPs from sequences; (v) tertiary analyses, including gene ontology and clustering of differences in expression and epigenetic status of genes; and (vi) quaternary analyses integrating across modalities. There are several potential solutions to these problems. Primary analyses of data acquired from DNA sequencing machines are usually performed on dedicated servers. For secondary analyses, fiber-optic connections to cluster servers and cloud computing networks with adequate storage (presently 30–100 terabytes) and automated data backup are mandatory. Tertiary analyses may be accomplished on cluster servers, cloud computing networks, and petaflop supercomputers.

Every individual's DNA is unique and can be potentially used as an identifier; therefore, measures need to be taken to ensure the privacy of the data collected from genetic studies. However, firewalls that are necessary for the security and confidentiality of patient data can impede access and sharing of data by researchers. Data enclaves in which information is not collected, banked, or shared but allow investigators to work with specific data sets once they have filed a research protocol regarding what can be printed, saved, and removed from the site is one method of sharing data sets while protecting the identity of participants. The transfer of large files is another frequent problem but can be addressed by high-speed links to nearby supercomputers and cloud computing systems. Availability of cheap and effective laboratory information management systems would also accelerate progress.

Clearly, collaborations among biologists, statisticians, and informatics technology specialists will be needed to best obtain, store, and interpret the massive amounts of data created. Courses in advanced software development and systems management would be integral to professional development. An important focus could be algorithm development in specialized areas, for example, hidden Markov chains, simulations, expectation-maximization algorithms, and statistical genetics.

Practical issues of implementing genetic testing

The use of genetic testing in medicine has been the subject of much debate. As the era of whole-genome sequencing for every individual draws near, legislation is required in order to protect individuals from potential misuse of the information. Recently, the US Senate unanimously passed the Genetic Information Nondiscrimination Act, which prohibits employers from inquiring about genetic testing or using an individual's genetic information as the basis for hiring, firing, or promoting.[49] The act also applies to health insurance plans; it prohibits the setting of eligibility, premium, or contribution amounts on the basis of genetics and also prohibits requiring an individual to take a genetic test.[49] A similar voluntary moratorium is also in operation in the United Kingdom. Other considerations include the disclosure of genetic information when it can benefit third parties, such as sharing an individual's genetic test result with their children; as discussed above, the security of stored genetic data needs to be ensured.

Another issue entails provision of funding and resources for genetic research related to addictions. Funding agencies for scientific studies vary across countries, and drug addiction is often not a high research priority, given the general belief that drug use can be controlled by the law (even though prohibition has not proven successful in the past), and many members of the general public still tend to view it as an issue of willpower. Therefore, there needs to be better education of the general public, health-care providers, and policy makers on how to interpret genetic information. The general public tends to view genes as deterministic, and it is important to improve the ability to convey risk, as opposed to absolutes, when disseminating information on diseases such as drug addiction, in which many other factors also contribute. Individuals also need to be protected against companies that attempt to profit from unsound or presently unvalidated genetic tests. The commercialization of genetic testing is well under way, with companies offering sequencing services alongside a list of diseases to which one is susceptible, for a price. There are also companies that claim to be able to optimize the treatment of addictions on the basis of genetics (e.g., Salugen, http://www.salugen.com, and NicoTest,

http://vcc.nicotest.com) and to predict the risk of developing lung cancer (http://www.respiragene.com). Such ventures need to be regarded with caution, especially given that the predictive power of genetic tests is still highly limited at best.

CONCLUSIONS

Despite the substantial consequences to the individual and society, genetic research on addictions has remained a relatively low priority. Recent technological advances have provided the tools to detect variations in the genetic architecture between individuals; however, much work still needs to be done to determine the biological relevance and to interpret the associations between these genetic variants and addiction-related phenotypes. An improvement in our understanding of the genetic and environmental factors underlying drug addiction has the potential to increase our understanding of the etiology and neurobiology of addictions, leading to greatly reduced morbidity and mortality by providing novel treatments and by improving the success rates of existing treatments.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

ACKNOWLEDGMENTS

This paper originated from an Esteve Foundation Discussion Group meeting, and the authors are grateful for the foundation's support. Additional support from the Centre for Addictions and Mental Health, an NSERC CGS-D scholarship, and a SPICE scholarship from the Interdisciplinary Capacity Enhancement Team to M.K.H. are acknowledged. A.H. receives support from the German Federal Ministry of Education and Research (National Genome Research Network, NGFNplus: 01GS08159). J.K. receives funding from the Academy of Finland Centre of Excellence in Complex Disease Genetics, Academy of Finland grants 118555 and DA012854. M.J.K. is in part supported by NIH-NIDA P60-DA005130, NIH-NIMH R01-MH79880, NIH UL1 RR024143, and R01 MH076537 (H. Crystal, principal investigator). M.D.L. is supported in part by DA012844 and DA013783. R.F.T. receives support from the Centre for Addictions and Mental Health, a Canada Research Chair in Pharmacogenetics, CIHR MOP86471, and NIH DA 0220830.

CONFLICT OF INTEREST

J.K. has served as a consultant to Pfizer. In order to receive compensation from the University of Virginia, M.D.L. has served as a consultant to the National Institutes of Health, DeCODE Genetics, the University of Pennsylvania, Reckitt Benckiser Pharmaceuticals, the Pennsylvania Department of Health, and Informational Managements Consulting. M.D.L. also serves as a scientific adviser to ADial Pharmaceuticals. R.F.T. holds shares in and is a CSO in Nicogen Research Inc., a company focused on novel approaches to smoking-cessation treatment. None of the data contained in this manuscript alters or improves any commercial aspect of Nicogen, no Nicogen funds were used in this work, and the manuscript was not reviewed by others affiliated with Nicogen. R.F.T. has also been a paid consultant for Novartis. R.F.T. is also an Associate Editor of *Clinical Pharmacology & Therapeutics*. She was not involved in the peer review or decision processes for this manuscript. M.K.H., D.G., A.H., M.J.K., and M.R.M. report no conflicts of interest.

1. World Health Organization. Substance Abuse Facts and Figures: The Global Burden, 2002 <http://www.who.int/substance_abuse/facts/global_burden/en/index.html>.
2. Office of National Drug Control Policy. *The Economic Costs of Drug Abuse in the United States, 1992–2002* (Washington, DC, 2004).
3. Goldman, D., Oroszi, G. & Ducci, F. The genetics of addictions: uncovering the genes. *Nat. Rev. Genet.* **6**, 521–532 (2005).
4. Ho, M.K. & Tyndale, R.F. Overview of the pharmacogenomics of cigarette smoking. *Pharmacogenomics J.* **7**, 81–98 (2007).
5. Rose, R.J., Broms, U., Korhonen, T., Dick, D.M. & Kaprio, J. Genetics of smoking behavior. In *Handbook of Behavior Genetics* 411–432 (Springer, New York, 2009).
6. Agrawal, A. & Lynskey, M.T. Are there genetic influences on addiction: evidence from family, adoption and twin studies. *Addiction* **103**, 1069–1081 (2008).
7. Li, M.D. & Burmeister, M. New insights into the genetics of addiction. *Nat. Rev. Genet.* **10**, 225–231 (2009).
8. Ross, S. & Peselow, E. The neurobiology of addictive disorders. *Clin. Neuropharmacol.* **32**, 269–276 (2009).
9. Khokhar, J.Y., Ferguson, C.S., Zhu, A.Z. & Tyndale, R.F. Pharmacogenetics of drug dependence: role of gene variations in susceptibility and treatment. *Annu. Rev. Pharmacol. Toxicol.* **50**, 39–61 (2010).
10. Pearson, T.A. & Manolio, T.A. How to interpret a genome-wide association study. *JAMA* **299**, 1335–1344 (2008).
11. Bierut, L.J. Convergence of genetic findings for nicotine dependence and smoking related diseases with chromosome 15q24-25. *Trends Pharmacol. Sci.* **31**, 46–51 (2010).
12. Yuferov, V., Nielsen, D., Butelman, E. & Kreek, M.J. Microarray studies of psychostimulant-induced changes in gene expression. *Addict. Biol.* **10**, 101–118 (2005).
13. Huang, W. & Li, M.D. Nicotine modulates expression of miR-140*, which targets the 3′-untranslated region of dynamin 1 gene (Dnm1). *Int. J. Neuropsychopharmacol.* **12**, 537–546 (2009).
14. Xu, Q., Huang, W., Payne, T.J., Ma, J.Z. & Li, M.D. Detection of genetic association and a functional polymorphism of dynamin 1 gene with nicotine dependence in European and African Americans. *Neuropsychopharmacology* **34**, 1351–1359 (2009).
15. Huang, W. & Li, M.D. Differential allelic expression of dopamine D1 receptor gene (DRD1) is modulated by microRNA miR-504. *Biol. Psychiatry* **65**, 702–705 (2009).
16. Renthal, W. & Nestler, E.J. Epigenetic mechanisms in drug addiction. *Trends Mol. Med.* **14**, 341–350 (2008).
17. Nielsen, D.A. *et al*. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. *Neuropsychopharmacology* **34**, 867–873 (2009).
18. Pandey, S.C., Ugale, R., Zhang, H., Tang, L. & Prakash, A. Brain chromatin remodeling: a novel mechanism of alcoholism. *J. Neurosci.* **28**, 3729–3737 (2008).
19. Hughes, J.R. *et al*. Concordance of different measures of nicotine dependence: two pilot studies. *Addict. Behav.* **29**, 1527–1539 (2004).
20. Strat, Y.L., Ramoz, N., Schumann, G. & Gorwood, P. Molecular genetics of alcohol dependence and related endophenotypes. *Curr. Genomics* **9**, 444–451 (2008).
21. Puls, I. *et al*. Synergistic effects of the dopaminergic and glutamatergic system on hippocampal volume in alcohol-dependent patients. *Biol. Psychol.* **79**, 126–136 (2008).
22. Filbey, F.M., Ray, L., Smolen, A., Claus, E.D., Audette, A. & Hutchison, K.E. Differential neural response to alcohol priming and alcohol taste cues is associated with DRD4 VNTR and OPRM1 genotypes. *Alcohol. Clin. Exp. Res.* **32**, 1113–1123 (2008).
23. Visscher, P.M. & Montgomery, G.W. Genome-wide association studies and human disease: from trickle to flood. *JAMA* **302**, 2028–2029 (2009).
24. Keskitalo, K. *et al*. Association of serum cotinine level with a cluster of three nicotinic acetylcholine receptor genes (CHRNA3/CHRNA5/CHRNB4) on chromosome 15. *Hum. Mol. Genet.* **18**, 4007–4012 (2009).
25. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J.A. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* **324**, 387–389 (2009).
26. Tapper, A.R. *et al*. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. *Science* **306**, 1029–1032 (2004).
27. Picciotto, M.R. *et al*. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391**, 173–177 (1998).
28. Xu, W. *et al*. Megacystis, mydriasis, and ion channel defect in mice lacking the alpha3 neuronal nicotinic acetylcholine receptor. *Proc. Natl. Acad. Sci. USA* **96**, 5746–5751 (1999).
29. Portugal, G.S. & Gould, T.J. Genetic variability in nicotinic acetylcholine receptors and nicotine addiction: converging evidence from human and animal research. *Behav. Brain Res.* **193**, 1–16 (2008).
30. Bierut, L.J. *et al*. Variants in nicotinic receptors and risk for nicotine dependence. *Am. J. Psychiatry* **165**, 1163–1171 (2008).
31. van der Zwaluw, C.S. & Engels, R.C. Gene-environment interactions and alcohol use and dependence: current status and future challenges. *Addiction* **104**, 907–914 (2009).

32. Heinz, A., Mann, K., Weinberger, D.R. & Goldman, D. Serotonergic dysfunction, negative mood states, and response to alcohol. *Alcohol. Clin. Exp. Res.* **25**, 487–495 (2001).
33. Chatterjee, N. & Wacholder, S. Invited commentary: efficient testing of gene-environment interaction. *Am. J. Epidemiol.* **169**, 231–233; discussion 234 (2009).
34. Igl, W. *et al.*; EUROSPAN Consortium. Modeling of environmental effects in genome-wide association studies identifies SLC2A2 and HP as novel loci influencing serum cholesterol levels. *PLoS Genet.* **6**, e1000798 (2010).
35. Goldman, D. & Ducci, F. The genetics of alcoholism and other addictive disorders. In *Human Genetics* 4th edn. (eds. Speicher, M.R., Antonarakis, S.E. & Motulsky, A.G.) (Springer, Berlin, Germany, 2010).
36. Brooks, P.J., Enoch, M.A., Goldman, D., Li, T.K. & Yokoyama, A. The alcohol flushing response: an unrecognized risk factor for esophageal cancer from alcohol consumption. *PLoS Med.* **6**, e50 (2009).
37. Mwenifumbo, J.C. & Tyndale, R.F. Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. *Pharmacogenomics* **8**, 1385–1402 (2007).
38. Kreek, M.J. & LaForge, K.S. Stress responsivity, addiction, and a functional variant of the human mu-opioid receptor gene. *Mol. Interv.* **7**, 74–78 (2007).
39. Bond, C. *et al*. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc. Natl. Acad. Sci. USA* **95**, 9608–9613 (1998).
40. Oslin, D.W. *et al*. A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology* **28**, 1546–1552 (2003).
41. O'Loughlin, J. *et al*. Genetically decreased CYP2A6 and the risk of tobacco dependence: a prospective study of novice smokers. *Tob. Control* **13**, 422–428 (2004).
42. Audrain-McGovern, J., Al Koudsi, N., Rodriguez, D., Wileyto, E.P., Shields, P.G. & Tyndale, R.F. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. *Pediatrics* **119**, e264–e274 (2007).
43. Johnston, L.D., O'Malley, P.M., Bachman, J.G. & Schulenberg, J.E. Various stimulant drugs show continuing gradual declines among teens in 2008, most illicit drugs hold steady (University of Michigan News Services, Ann Arbor, MI, 11 December 2008).
44. Kreek, M.J., Zhou, Y., Butelman, E.R. & Levran, O. Opiate and cocaine addiction: from bench to clinic and back to the bench. *Curr. Opin. Pharmacol.* **9**, 74–80 (2009).
45. Doehring, A. *et al*. Genetic variants altering dopamine D2 receptor expression or function modulate the risk of opiate addiction and the dosage requirements of methadone substitution. *Pharmacogenet. Genomics* **19**, 407–414 (2009).
46. Haile, C.N., Kosten, T.A. & Kosten, T.R. Pharmacogenetic treatments for drug addiction: alcohol and opiates. *Am. J. Drug Alcohol Abuse* **34**, 355–381 (2008).
47. Heitjan, D.F., Asch, D.A., Ray, R., Rukstalis, M., Patterson, F. & Lerman, C. Cost-effectiveness of pharmacogenetic testing to tailor smoking-cessation treatment. *Pharmacogenomics J.* **8**, 391–399 (2008).
48. Welton, N.J., Johnstone, E.C., David, S.P. & Munafò, M.R. A cost-effectiveness analysis of genetic testing of the DRD2 Taq1A polymorphism to aid treatment choice for smoking cessation. *Nicotine Tob. Res.* **10**, 231–240 (2008).
49. Dressler, L.G. & Terry, S.F. How will GINA influence participation in pharmacogenomics research and clinical testing? *Clin. Pharmacol. Ther.* **86**, 472–475 (2009).
50. Heath, A.C. & Martin, N.G. Genetic influences on alcohol consumption patterns and problem drinking: results from the Australian NH&MRC twin panel follow-up survey. *Ann. NY Acad. Sci.* **708**, 72–85 (1994).